AD-A253 895

(2)

CHEMICAL RESEARCH, DEVELOPMENT & ENGINEERING CENTER

CRDEC-TR-363

ACUTE INHALATION TOXICITY EFFECTS OF EXPLOSIVELY DISSEMINATED -- XM82 GRENADE -- TITANIUM DIOXIDE

Roger J. Hilaski
Jeffrey D. Bergmann
John C. Carpin
William T. Muse, Jr.
Sandra A. Thomson

RESEARCH DIRECTORATE

June 1992

Approved for public release; distribution is unlimited.

U.S. ARMY ARMAMENT MUNITIONS CHEMICAL COMMAND

Aberdeen Proving Ground, Maryland 21010-5423

Disclaimer

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188

1. AGENCY USE ONLY *(Leave blank)*

2. REPORT DATE
1992 June

3. REPORT TYPE AND DATES COVERED
Final, 90 Aug - 91 Jan

4. TITLE AND SUBTITLE
Acute Inhalation Toxicity Effects of Explosively Disseminated -- XM82 Grenade -- Titanium Dioxide

5. FUNDING NUMBERS
PR-1L162622A552

6. AUTHOR(S)
Hilaski, Roger J., Bergmann, Jeffrey D., Carpin, John C., Muse, William T., Jr., and Thomson, Sandra A.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
CDR, CRDEC, ATTN: SMCCR-RST-E, APG, MD 21010-5423

8. PERFORMING ORGANIZATION REPORT NUMBER
CRDEC-TR-363

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Titanium dioxide (TiO_2) is the major component for a proposed training smoke grenade (XM82). The American Conference of Governmental Industrial Hygienists has classified TiO_2 as a "nuisance dust" with threshold limit value of 10 mg/m^3 of total dust (<1% quartz). Adverse pulmonary effects from TiO_2 occur only when lung clearance mechanisms are overwhelmed. The purpose of this study was to determine acute toxicological effects in rats exposed by inhalation to explosively disseminated TiO_2 and the resultant by-products present in the aerosol. Groups of male, Fisher 344, rats were exposed by nose-only inhalation to three concentrations of TiO_2 dust for 30 min. The highest concentration was used as the "worst case" field concentration. Two other concentrations were selected to produce a "no adverse effect" level. Air-exposed and fuse/fuel exposed rats served as controls. Exposed rats and respective groups of controls were submitted for bronchoalveolar lavage, biochemical, physiological, and pathological evaluations at 24-hr and 14-days post exposure. The results indicate there were no irreversible adverse changes in the biological response of the rats exposed to TiO_2 from the XM82 grenade.

14. SUBJECT TERMS
Titanium Dioxide (TiO_2) Inhalation
Dust XM82 Grenade

15. NUMBER OF PAGES
37

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

Blank

PREFACE

The work described in this report was authorized under Project No. 1L162622A552, Smoke and Obscurants. This work was started in August 1990 and completed in January 1991. The experimental data are recorded in laboratory notebooks 85-0162, 87-0065, 88-0117, 89-0171, 90-0094, and 91-0044.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. This study was consistent with Good Laboratory Practices and was conducted in accordance with Protocol No. 22091000A265 approved by the U.S. Army Chemical Research, Development and Engineering Center (CRDEC) Laboratory Animal Use Review Committee.

The use of trade names or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

Acknowledgments

The authors thank Charles Crouse for his help in the chamber set-up, Joseph Affleck and Joseph Hill for animal care and transportation, and Bernardita Infiesto and Dave Burnett for analysis of the lavage fluid, all of the Toxicology Division, CRDEC. The authors also thank Dottie Berg, Toxicology Division, CRDEC, for her assistance in manuscript preparation.

Blank

CONTENTS

LIST OF TABLES

ACUTE INHALATION TOXICITY EFFECTS
OF EXPLOSIVELY DISSEMINATED -- XM82 GRENADE -- TITANIUM DIOXIDE

1. INTRODUCTION

The chemical industry has developed an extensive data base on titanium dioxide (TiO_2) from its use in paint, paper, rubber, plastic, ceramics, inks, and floor coverings. The American Conference of Governmental Industrial Hygienists (ACGIH) has classified TiO_2 as a "nuisance dust" with a threshold limit value (TLV) of 10 mg/m^3 of total dust (<1% quartz).[1] The nuisance dust category has been confirmed by numerous inhalation studies.[2-5] There has been no epidemiological evidence of adverse pulmonary effects in workers occupationally exposed to TiO_2 for over 10 years.[6] Some adverse pulmonary effects from exposure to TiO_2 occur only when lung clearance mechanisms are overwhelmed.[4,7]

Titanium dioxide is the proposed major component for a training grenade -- XM82 -- under development by the U.S. Army Chemical Research, Development and Engineering Center (CRDEC). The military use of TiO_2 is a unique application requiring additional evaluation. This study was designed to determine the acute inhalation toxicological effects in rats exposed to explosively disseminated TiO_2 and the resultant by-products present in the aerosol. The purpose was to mimic actual field concentrations under controlled conditions.

2. MATERIALS AND METHODS

2.1 Materials.

Three TiO_2 dusts from different sources have been tested in a nonexplosively generated inhalation study.* There were no significant adverse toxicological effects from any of the dusts.* The dusts were approximately 97% TiO_2 and contained variable amounts of amorphous silica, alumina or aluminum oxide (<2%), and siloxane hydrophobic coating (<0.5%). All three materials were the rutile form of TiO_2. Tioxide America, Incorporated, was the source for TiO_2 (R-FC6) used in the grenades used in this study. The XM82 grenades were prepared by the Munitions Directorate, CRDEC, and were composed of the following items:

- Expulsion charge (Propellant - Class 6 black powder)
- Delay detonator assembly

Carpin, J.C., Hilaski, R.J., Burnett, D.C., Bergmann, J.D., and Thomson, S.A., Comparative Inhalation Toxicity of Three Hydrophobically Coated Titanium Dioxide Powders, U.S. Army Chemical Research, Development and Engineering Center, Aberdeen Proving Ground, MD, unpublished data.

- Composition A-1A
- Lead azide
- RDX, Type B
- Zirconium/nickel delay

- Transfer lead (PBXN-5)
- Booster lead (Composition CH-6)
- Burster (Composition A-5) (dispersed the TiO_2)
- TiO_2

Detailed descriptions of XM82 grenade components are listed in Appendix A of this report.

2.2 Animal Utilization/Husbandry/Necropsy.

Male, Fisher 344, rats were purchased from the Charles River Laboratories (Wilmington, MA). On arrival, the rats were housed in individual, suspended stainless steel cages in the Toxicology Division, CRDEC, animal care facility. Housing conditions were 12-hr light/dark cycle with 20-24 °C temperature and 30-70% relative humidity. Water and certified rodent chow were provided ad libitum. Cage and tray cleaning were performed three times weekly. Rodent management, handling, and utilization were in accordance with the National Institute of Health Publication 85-23, "Guide for Care and Use of Laboratory Animals."[8] Daily observations for aberrant behavior and toxic signs, along with weekly weighings, were made throughout the study. During the standard quarantine period of 7 days, the animals were examined by the Chief, Veterinary Services Branch, CRDEC, and determined to be in good health (Appendix B of this report).

One week prior to the scheduled start date, the rats were weighed, tattooed, and randomly placed into groups. On the day of exposure, a climate-controlled vehicle was used to transport the rats to the exposure chamber, and they were returned to the animal facility immediately after exposure.

NUMBER OF RATS

	Pathology		Lavage and Physiology	
	24-Hr PE*	14-Day PE	24-Hr PE	14-Day PE
Controls	6	6	6	6
Fuse/Fuel	6	6	6	6
Low Concentration	6	6	6	6

	Pathology		Lavage and Physiology	
	24-Hr PE*	14-Day PE	24-Hr PE	14-Day PE
Mid Concentration	6	6	6	6
High Concentration	6	6	6	6

*Post Exposure (PE)

Since pathological evaluation requires that the animals be terminated, separate animal groups were needed for pathology and lavage/ physiology. However, bronchoalveolar lavage (BAL) and physiology measurements were conducted on the same animal. Groups of 12 male rats were exposed by nose-only inhalation to three concentrations of TiO_2 dust for 30 min. Exposed rats and respective groups of air and fuse/fuel exposed controls were evaluated for physiological, BAL, and histopathological changes at 24-hr and 14-day PE. Previous studies with pyrotechnic/explosive devices have shown a significant amount of particulate and vapor phase contributions from the fuse/fuel components.[9,10]

2.3 Chamber Operation and Sample Collection.

The rats were exposed in a 250-L, nose-only chamber that was connected to a 20-m^3 chamber via a 6-in. diameter duct. One-quarter size grenades were detonated in the 20-m^3 chamber, and the resulting aerosol cloud was used for the exposures in the 250-L chamber. The aerosol cloud was inside the 20-m^3 was continuously stirred to keep the particles suspended. Airflow through the 250-L chamber was 500 L/min for all exposures. The atmosphere in the 20-m^3 chamber was conducted through a 2-in. diameter PVC pipe (which extended to the middle of the chamber) to the nose-only chamber. The low concentration was attained by using an orifice cap with a 3/4-in. diameter hole at the end of the PVC pipe and 50% dilution with room air. The 50% dilution with room air without the orifice cap was used for the mid concentration. The aerosol cloud direct from the large chamber without dilution or restriction was used for the high concentration. During chamber exposure, concentration was measured gravimetrically by drawing chamber air through glass-fiber filter pads. An initial measurement of the 20-m^3 chamber atmosphere was taken 2 min after grenade detonation. Filter pad samples of the nose-only chamber atmosphere were taken at 5-, 15-, and 25-min intervals after the animal exposure began. Also, a continuous sample for the entire animal exposure was collected. This was used to determine a time-weighted average (TWA) concentration. Particle size samples were taken with an Anderson Instruments (Atlanta, GA) Cascade Impactor Model 2210. Air from both chambers was exhausted through particulate filters.

Combustion gases were monitored using Matheson-Kitagawa (East Rutherford, NJ) Precision Gas Detector Tubes. Samples for analysis were drawn at two discrete intervals (5 and 20 min) during exposure. At each interval, 2 L of combustion gases was drawn from the 250-L chamber using a 2-L syringe. A calibrated 100 mL Matheson-Kitagawa sampling pump collected specified volumes of gas through the detector tubes. After waiting the specified gas reaction time, colorimetric indicators inside the tubes indicated the concentration of the specific gas. The gases analyzed at the highest TiO_2 concentration and found to be below detectable limits were SO_2 [<1.0 parts per million (ppm)], NO_2 (<0.25 ppm), and methyl chloroform (CH_3CCl_3) (<15 ppm). The gases routinely analyzed during all exposures were carbon monoxide (CO), carbon dioxide (CO_2), nitrogen oxide (NO_x), and formaldehyde (HCHO).

2.4 Physiological Measurements.

Respiratory physiological testing and bronchoalveolar lung lavage were performed on the same animal. At 24-hr and 14-day PE, rats were anesthetized with sodium pentobarbital (40 mg/kg) by intraperitoneal injection. Two respiratory parameters, flow and transpulmonary pressures, were determined directly from each animal. Respiratory flow was measured through a tracheal catheter connected to a Fleisch (Dynasciences, Blue Bell, PA) pneumotachometer. A Validyne (Validyne Engineering Corporation, Northridge, CA) differential pressure transducer, attached to the pneumotachometer by tubing, converts the flow to the proper signals for the Buxco Pulmonary Mechanics Analyzer (Buxco Electronics, Incorporated, Troy, NY).

For transpulmonary pressure, a catheter was inserted into the esophagus approximately to the level of the thoracic inlet. The catheter was connected to one arm of a Statham (Statham Laboratories, Incorporated, Hato Rey, Puerto Rico) differential pressure transducer. Transpulmonary pressure (taken as the difference between esophageal pressure and airway pressure derived from a lateral tap at the distal end of the endotracheal tube) was used for all calculations.

The flow and pressure signals were processed in a Buxco Pulmonary Mechanics Analyzer. The parameters recorded from the analyzer were the flow, transpulmonary pressure, tidal volume, compliance, resistance, respiratory rate, and minute volume.

Compliance is a standard physiological method of assessing the overall lung and thoracic elasticity. Compliance is measured as the ratio of the tidal volume to the pressure change between end expiration and end inspiration. Restrictive pulmonary diseases (e.g., fibrosis and silicosis) result in decreases in compliance due to a stiffening effect that increases the work of breathing. Resistance is a measure of the pressure difference required per unit of flow change. Inhalation of dusts/fibers may lead to an increase in airway resistance. Both compliance and resistance were measured as indicators of functional impairment.

2.5 BAL.

Immediately following the pulmonary measurements, the esophageal catheter was withdrawn, and the lavage procedure was started. The lung washing technique consisted of introducing a volume of saline (0.015 mL/g body weight) into the lung and immediately withdrawing the saline until a slight pressure was noticed on the syringe plunger (VP Series II Bichromatic Analyzer, Abbott Laboratories, Irving, TX). Two lung washings were done in this manner in rapid succession. The lung washings were combined and centrifuged at 300 g for 10 min at 4 °C.

Following centrifugation, the supernatant fluid was separated from the pellet. The pellet was resuspended in 1 mL 50% bovine serum albumin, and total cell counts were taken on a ZBI (Hialeah, FL) Coulter Counter. A differential cell count was made using a modified Pap staining method (a common name for Papanicolaou's test). The cell pellet was resuspended in Hank's buffered saline, the macrophage concentration was determined in a hemocytometer, and the cell viability was determined via the trypan blue exclusion test.[11]

The supernatant lavage fluid was assayed for total protein and enzymatic activity of lactate dehydrogenase (LDH), alkaline phosphatase (ALKP), and B-Glucuronidase (B-Glu). The LDH and ALKP were determined on an Abbott VP Series II using an Abbott Analysis Kit, and B-Glu was assayed using a Sigma Chemical Company (St. Louis, MO) kit.

2.6 Histopathology.

At appropriate intervals, rats scheduled for pathology were euthanized with carbon dioxide and then necropsied. The total body weight and selected organ weight (adrenals, brain, kidneys, liver, lungs, testes) were recorded as part of the necropsy, and their tissues prepared for light microscope examination by Pathology Associates, Incorporated (Frederick, MD), in accordance with Contract No. DAAA15-88-D-0012. Pathology Associates, Incorporated, evaluated the tissues for histopathologic deviations.

2.7 TiO_2 Determination.

The actual amount of TiO_2 collected on the glass-fiber filter pads was determined by atomic absorption (AA) analysis for titanium (Ti) with subsequent conversion to TiO_2. This was done to verify that the majority of aerosol particles in the exposure atmosphere consisted of TiO_2 and not other grenade by-products. In addition, the test material [TiO_2 (R-FC6)] used for the study was analyzed to confirm the specified 97% purity for TiO_2.

Two sets of filter pads were analyzed. The first set was the 45-mm, glass-fiber filters used to collect the TWA samples from each exposure. Whereas, the second set consisted of 25-mm filters that were used to collect the serial samples at specified intervals during the exposure. A select number of these samples were assayed, and the means and standard deviations were calculated.

The filter pads were acid digested with a mixture of 10 mL hydrofluoric acid +5 mL nitric acid (HNO_3). All filter pads contained <0.1 g of powdered material. Each filter and acid were added together in a Teflon digestion vessel, and the vessel closed to a torque of 12 ft/lb. Four sample vessels were loaded into the microwave turntable (CEM Corporation, Matthews, NC) and spaced at 90° from each other. The microwave oven was operated for 15 min at 55% heating power. This digestion resulted in complete destruction of the filter matrix as well as complete solubilization of the TiO_2 material. After digestion and cooling, the contents were quantitatively transferred to Nalgene volumetric containers. All final dilutions and aqueous Ti standards contained 0.1% of ionization suppressant. Titanium analysis by AA used a nitrous oxide flame. Filter blanks and weighed TiO_2 powder were tested concurrently with the sample filters to establish the absence of interferences and the percent recovery of the method.

2.8 Data Analysis Plan.

Data analysis was conducted according to the statistical "decision tree" described by Gad and Well.[12] Bartlett's Test for Homogenicity of Variance was used as a check of the analysis of variances (ANOVA) assumptions, followed by the ANOVA. Nonparametric data was analyzed by the Kruskal-Wallis nonparametric ANOVA.

This study was consistent with Good Laboratory Practices. Every reasonable attempt was made to control bias throughout the experiment. All chamber analysis data, toxic observations, and animal weights were recorded in official CRDEC notebooks. Physiology and lavage data were generated on hard copy outputs from automated instruments. These data were entered into and analyzed in official CRDEC notebooks. All other associated raw data (statistical printouts, necropsy incidence tables, etc.) were stored in the Research Directorate, Toxicology Division, CRDEC, archives.

3. RESULTS

3.1 Chamber Analysis.

As expected, the downsized grenades produced a very dense white cloud in the 20-m^3 chamber. Two minutes after the grenade detonation, a filter pad sample from the large chamber was taken to provide guidance for sampling times in the 250-L chamber. The aerosol concentration measurements recorded from each exposure day are in Table 1 along with the results of the combustion gas monitoring and the 20-m^3 chamber concentration. The airflow rate through the 250-L, nose-only chamber was maintained at 500 L/min. In addition to the continuous TWA, gravimetric samples were collected at 5-, 15-, and 25-min intervals from the start of animal exposure to show the rate of decline of the particle concentration and as an additional verification of the nose-only chamber concentration.

3.2 Particle Size Analysis.

The aerodynamic particle size of the aerosol was determined using an Anderson Instruments (Atlanta, GA) Cascade Impactor Model 2210-K, 10-stage (Table 2). The cascade impactor was operated at 0.25 CFM, and the sample time varied with the concentration. At this flow rate, the particle size could be measured between 18 and 0.9 μm. Test material was collected on lightly greased, stainless steel substrates that were weighed prior to and following sampling to determine the mass collected in each range size. Particle size sample data were analyzed by log-normal regression, least squares method, of particle size versus cumulative relative mass. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (σ_g) of the aerosol was determined during the calibration and exposure phases of the study.

3.3 Toxic Sign Observations.

Rats exposed to 30 min of explosively disseminated TiO_2 did not exhibit any adverse toxic effects. Nose-only exposure devices have the inherent disadvantage of severely reducing the natural movement and visibility of test animals. The rats did not appear to be over heated, stressed, or uncomfortable. All exposed rats recovered from the cramped conditions within 1 hr of exposure. Both controls and exposed rats displayed similar behavior on removal from the holders. All exposed rats gained weight at the same rate as control animals.

3.4 Physiological and Bronchoalveolar Responses.

The results of the physiological and BAL evaluations are in Tables 3, 4, and 5. There were no statistically significant differences in the physiology or BAL parameters between exposed and control rats.

3.5 Pathological Evaluations.

The administration of explosively disseminated TiO_2 dust resulted in the deposition of brown granular pigment in the lungs of all exposed rats. The pigment was contained within macrophages. No toxic or inflammatory reactions, other than phagocytosis of the dust particles in the lung, were present. Pigment-laden macrophages were also present in the lungs of all rats in the fuse/fuel-exposed control group. There were no other significant gross or histopathologic differences between the animals in the 24-hr and 14-day PE groups.

Multifocal, centrilobular, hepatocellular, and cytoplasmic vacuolization was noted in many of the rats. No relationship to the treatment was apparent. This change probably reflected the presence of glycogen and was not considered a significant pathologic change.

Respiratory epithelial, hyperplasia was present in at least one rat from each dose group, except in the low dose sacrificed at 24-hr PE, and in at least two rats from each dose sacrificed at 14-day PE. No relationship to the treatment was apparent. This change was most likely due to exposure to an unknown toxic or infectious agent.

Microscopically, the only significant treatment-related finding was the presence of pigment (primarily within macrophages) in the alveoli and small airways of rats exposed to both TiO_2 and fuse/fuel-necropsied at 24-hr and 14-day PE and in the tracheobronchial lymph nodes of three out of six high-dose rats necropsied at 14-day PE. No other significant morphologic changes were present. The summary report from Pathology Associates is in Appendix C of this report. All original data are stored in the Toxicology Division, Research Directorate, CRDEC archives.

3.6 TiO_2 Content.

The results from four samples of the test material (R-FC6) used in this study met the manufacturer's specification of 97 ± 0.8% TiO_2. The results of the filter pad analysis verified that the majority of the exposure atmosphere consisted of TiO_2 dust. The mean percent TiO_2 recovery from five TWA filter pads was 91 ± 4.8%. Mean percent recovery from eight serial filter pads was 104 ± 2.8%. These values were adjusted for the 97% purity of the test material and represent the percentage of the AA filter concentration divided by the gravimetric aerosol concentration.

4. DISCUSSIONS

Groups of male, Fischer 344, rats were exposed to three concentrations of explosively disseminated TiO_2 dust for 30 min. Additional control groups of rats were exposed to either air or fuse/fuel components. At 24-hr and 14-day PE, the rats were evaluated for pulmonary mechanics, BAL, and histopathological changes. There were no compound-related mortalities and no significant changes in pulmonary mechanics, BAL, or histopathology.

CO, CO^2, NO_x, HCHO, and CH_3CCl_3 were measured during each exposure. With the exception of CO and HCHO (24-hr PE fuse/fuel and high concentration groups only), the levels of CO_2, NO_x, and CH_3CCL_3 were below the 5,000-, 3-, and 350-ppm TLV, respectively, and below the short-term exposure levels (STEL) of 30,000, 5, and 450 ppm, respectively, established by the American Conference of Governmental Industrial Hygienists -- 1990. The CO was below the 50-ppm TLV-TWA and the STEL of 400 ppm in each exposure except the fuse/fuel group (24-hr PE sacrifice) where the average level was 52 ppm. The HCHO levels were slightly more variable. The HCHO TLV-TWA was 1 ppm, and the STEL was 2 ppm. The average levels of 3 and 2.5 ppm were in the fuse/fuel and high concentration groups (24-hr PE sacrifice), respectively, with the other exposures at or below the STEL. These levels of HCHO may not be relevant because these tests were conducted in a closed chamber. Whereas, actual field disseminations would probably show a negligible amount of HCHO present in the open atmosphere.

The decision to use a quarter-size grenade was based on two factors. First, for comparison purposes, we wanted the low concentration to approximate concentrations from previous studies with TiO_2. Second, to approximate field conditions, it was desirable to have the highest possible concentration (without altering the particle size) for the high concentration.

Two recent studies used 100 mg/m^3 in repeated acute inhalation evaluations;[*,5] therefore, we used this level as our target for the low concentration.

A full-size grenade was discharged in the 20-m^3 chamber during calibration and after animal exposures. The resulting aerosol cloud was drawn into the 250-L chamber in the same manner as the high concentration exposures.

The same parameters were measured with the following results:

	20-m^3 Chamber	250-L Chamber
MMAD (μm)	2.97	2.51 (+8 min)
	-	2.57 (+25 min)
σ_g	3.66	3.49 (+8)
		3.37 (+25)
Aerosol	8.57 (+6 min)	4.8 (+5 min)
Conc (gm/m^3)	-	3.5 (+15 min)
	-	1.8 (+25 min)
CO (ppm)	-	130
CO_2 (ppm)	-	550
NO_x (ppm)	-	0.15
HCHO (ppm)		<2
CH_3CCl_3 (ppm)		<10

Clearly, the combustion gases from the full-sized grenade were either at or below the published STELs.

Based on this information, we decided to use a quarter-size grenade without air dilution for the high level, and 50% air dilution for the mid-level concentration targeting for 1,000 mg/m^3. We recognized that the concentrations would be continuously decreasing throughout exposure. Chamber concentrations outlined in Table 1 of this report were extremely variable. This was due in part to the unique method of dissemination. Despite taking every precaution to ensure that every grenade was identical, a certain degree of unpredictability remained when they were exploded. This made it difficult to ensure that each aerosol cloud would be identical to the others. Also, improved engineering controls would enhance the reproducibility of animal exposure.

None of the rats from the high concentration groups exhibited any pulmonary lung lesions or significant morphologic changes, thereby demonstrating that even these high-level, short-term exposures did not overwhelm the lung clearance mechanisms.

Carpin, J.C., Hilaski, R.J., Burnett, D.C., Bergmann, J.D., and Thomson, S.A., Comparative Inhalation Toxicity of Three Hydrophobically Coated Titanium Dioxide Powders, U.S. Army Chemical Research, Development and Engineering Center, Aberdeen Proving Ground, MD, unpublished data.

5. CONCLUSIONS

Thirty-minute, nose-only inhalation exposure of the rats to TiO_2 dust disseminated from quarter-size XM82 training grenades produced no adverse pulmonary affects. Short-term, high-level exposures do not appear to be harmful, but repeated dust exposures should be kept below the American Conference of Governmental Industrial Hygienists nuisance dust level ($10 mg/m^3$).

This method of dissemination and animal exposure is a viable alternative to the typical dust generation type of exposure techniques. Explosive/pyrotechnic grenade dissemination yields an aerosol that is very similar to actual field conditions. Different sized grenades, including full-size smoke grenades, could be used for specific, short-term investigations.

Table 1. Chamber Concentrations From Acute Inhalation Exposures (30 Min) Using the XM82 Grenade for Dissemination.

Chamber Concentrations From Acute Inhalation Exposures (30 Min)

24-Hr PE:

	Control	Fuse/Fuel	Low Conc	Mid Conc	High Conc
20,000-L Chamber (gm/m^3)	-	2.3	2.8	3.2	
TiO_2/Aerosols (mg/m^3)					
TWA*	-	115	38	351	830†
Conc +5**	-	-	-	695	2,260
+15	-	-	50	354	1,506
+25	-	-	48	274	1,000
CO (ppm)***	<2	52	<0.5	11	40
CO_2 (ppm)***	600	850	600	550	750
NO_x (ppm)***	-	1.6	<0.25	0.5	3.7
HCHO (ppm)***		3	<0.5	2.0	2.5
CH_3CCl_3 (ppm)***	-	-	-	-	12.5

14-Day PE:

	Control	Fuse/Fuel	Low Conc	Mid Conc	High Conc
20,000-L Chamber (grams TiO_2/m^3)			1.9	2.5	2.9
TiO_2/Aerosols (mg/m^3)					
TWA*	-	72	119	870	1,240
Conc +5**	-	-	160	1,080	1,960
+15	-	-	119	744	1,300
+25	-	-	116	563	964
CO (ppm)***	<2	35	7	32.5	47.5
CO_2 (ppm)***	875	875	650	700	950
NO_x (ppm)***	-	1.95	<0.05	0.7	0.95
HCHO (ppm)***		<2	<1	<2	2
CH_3CCl_3 (ppm)***		-	-	-	<10

*TWA concentration for 30-min exposure

**Gravimetric samples taken at 5-, 15-, and 25-min intervals after start of animal exposure

***Average of 2 samples taken at 5- and 20- min intervals after start of animal exposure with Matheson gas sample tubes

†Questionable results, sampling error

Table 2. Particle Size Data.

Particle Size Data						
	24-Hr PE			14-Day PE		
	Low Conc	Mid Conc	High Conc	Low Conc	Mid Conc	High Conc
MMAD	2.24	2.00	-	2.08	2.16	2.19
σ_g	3.28	3.42	-	3.37	3.42	3.30

MMAD - Mass Median Aerodynamic Diameter in Micrometers
σ_g - Geometric Standard Deviation

Table 3. Lung Lavage* Fluid Analysis Results From Rats Exposed to Grenade Disseminated TiO_2.

Lung Lavage* Fluid Analysis Results**				
	B-Glu (Sigma U/mL)	LDH (IU/L)	Alk Phos (IU/L)	Protein (µg/mL)
24-Hr PE:				
Control	4.15	52.93	79.3	317
	0.62	23.90	12.8	66
Fuse/Fuel	4.55	55.62	74.3	262
	0.68	29.81	6.1	63
Low Conc	4.82	50.88	64.9	342
	0.52	23.45	9.8	106
Mid Conc	4.57	57.98	71.8	328
	0.43	19.13	13.1	72
High Conc	4.47	38.42	60.3	345
	0.58	11.42	11.6	53
Bartlett's Test	NS	NS	NS	NS
ANOVA	NS	NS	NS	NS

Table 3. Lung Lavage* Fluid Analysis Results From Rats Exposed to Grenade Disseminated TiO_2 (Continued).

Lung Lavage* Fluid Analysis Results**				
	ß-Glu (Sigma U/mL)	LDH (IU/L)	Alk Phos (IU/L)	Protein (µg/mL)
14-Day PE:				
Control	4.05	52.75	92.11	484
	0.24	21.43	8.50	179
Fuse/Fuel	4.41	50.72	91.60	322
	0.76	26.65	10.79	60
Low Conc	4.31	60.15	94.05	337
	0.57	17.51	13.93	5
Mid Conc	3.75	50.22	79.68	332
	0.43	18.29	6.71	92
High Conc	4.12	57.50	87.95	307
	1.23	23.91	8.87	48
Bartlett's Test	SIG	NS	NS	SIG
ANOVA	-	NS	NS	-
Kruskal-Wallis Nonparametric ANOVA	NS	-	-	NS

*BAL
**Mean ± Standard Deviation, n=6
SIG = Significant
NS = No Significance

Table 4. Cytological Summary of Lavage Fluid From Rats Exposed to Grenade Disseminated TiO_2.

	WBC*,*** ($x10^3$)	Total*** ($x10^4$)	Macrophages (%)	Lymphocytes (%)	PMN**,*** (%)
24-Hr PE:					
Control	1.24	4.57	99	1	0
	0.62	2.43	2	1	0
Fuse/Fuel	1.90	4.48	99	1	0
	0.44	1.04	3	0	0
Low Conc	3.02	4.55	98	2	0
	0.70	0.82	2	1	0
Mid Conc	1.88	3.94	99	1	0
	0.26	0.84	1	1	0
High Conc	1.72	4.34	97	2	1
	0.61	1.59	2	1	1
Bartlett's	NS	NS	-	-	-
ANOVA	NS	NS	-	-	-
14-Day PE:					
Control	1.57	2.75	94	1	5
	0.23	1.55	5	1	5
Fuse/Fuel	2.02	2.07	96	2	2
	0.59	0.36	2	2	1
Low Conc	1.73	2.05	97	2	2
	0.28	0.25	2	1	0
Mid Conc	1.72	1.77	97	2	1
	0.63	0.50	2	1	1
High Conc	1.15	2.05	96	1	3
	0.52	0.98	2	1	1
Bartlett's	NS	NS	-	-	-
ANOVA	NS	NS	-	-	-

*White Blood Cell
**Polymorphonuclear Neutrophils
***Mean ± Standard Deviation, n=6
SIG = Significant
NS = No Significance

Table 5. Respiratory Physiology Data From Acute Inhalation of Explosively Disseminated TiO_2

Respiratory Physiology Data								
Group (n=6)	Weight (g)	Flow	Pleur. Press.	Tidal Vol.	Com-pliance	Resis-tance	Rest. Rate	Min. Vol.
24-Hr PE:								
Control	215	19.1	10.30	1.73	0.191	0.189	87.2	160
SD ±	7	4.0	2.03	0.32	0.072	0.061	17.4	44
Fuse/Fuel	217	20.1	9.77	1.88	0.212	0.168	96.5	198
SD ±	6	4.3	2.24	0.23	0.062	0.065	9.3	37
Low Conc	207	17.6	9.22	1.64	0.195	0.205	117.6	214
SD ±	3	3.5	1.00	0.21	0.048	0.098	15.4	41
Mid Conc	212	18.1	9.02	1.69	0.200	0.163	117.4	211
SD ±	5	3.6	1.00	0.28	0.062	0.039	33.6	42
High Conc	212	19.7	10.40	1.63	0.166	0.144	105.8	189
SD ±	NS	1.3	1.69	0.26	0.042	0.036	9.3	38
Bartlett's	SIG	NS	NS	NS	NS	NS	SIG	NS
ANOVA	NA	NS	NS	NS	NS	NS	NA	NS
Kruskal-Wallis Nonparameteric ANOVA	NS	-	-	-	-	-	NS	-
14-Day PE:								
Control	252	19.0	9.03	2.07	0.317	0.199	116.1	256
SD±	17	4.4	1.34	0.22	0.146	0.047	29.2	54
Fuse/Fuel	252	20.8	8.48	1.85	0.251	0.161	103.5	204
SD ±	7	1.7	1.74	0.10	0.051	0.058	19.1	39
Low Conc	240	18.7	6.83	1.97	0.333	0.128	101.0	210
SD ±	9	4.1	1.44	0.32	0.092	0.042	20.4	33
Mid Conc	247	19.8	7.75	1.87	0.286	0.148	102.9	207
SD ±	4	3.3	1.26	0.16	0.077	0.030	19.3	34
High Conc	245	19.4	8.26	2.04	0.331	0.196	101.9	220
SD ±	13	4.9	2.04	0.35	0.139	0.064	17.5	18
Bartlett's	SIG	NS	NS	NS	NS	NS	NS	NS
ANOVA	NA	NS	NS	NS	NS	NS	NS	NS
Kruskal-Wallis Nonparametric ANOVA	NS	-	-	-	-	-	-	-

SIG = Significant
NS = No Significance

Blank

LITERATURE CITED

1. Guide to Occupational Exposure Values, American Conference of Governmental Industrial Hygienists, Incorporated, Cincinnati, OH, 1990.

2. Ferin, J., and Oberdorster, G., "Biological Effects and Toxicity Assessment of Titanium Dioxides," Am. Ind. Hyg. Assoc. J. Vol. 46, pp 69-72 (1985).

3. Higgins, J., Driscoll, K.E., Lindenschmidt, Mauer J., and Perkins, M., "Pulmonary Responses to a 5 Day Inhalation Exposure to Silica or Titanium Dioxide," Toxicology Vol. 10, p 277 (1990).

4. Lee, K.P., Henry, N.W., Trochimowicz, H.J., and Reinhart, C.F., "Pulmonary Response to Impaired Lung Clearance in Rats Following Excessive TiO_2 Dust Deposition," Environ. Res. Vol. 41, pp 144-167 (1986).

5. Thomson, S.A., Bergmann, J.D., Burnett, D.C., Carpin, J.C., Crouse, C.L., Hilaski, R.J., Infiesto, B., and Lawrence-Beckett, E., Comparative Inhalation Screen of Titanium Dioxide and Graphite Dusts, CRDEC-TR-88161, U.S. Army Chemical Research, Development and Engineering Center, Aberdeen Proving Ground, MD, November 1988, UNCLASSIFIED Report.

6. Chen, L., and Fayerweather, W.E., "Epidemiologic Study of Workers Exposed to Titanium Dioxide," J. Occup. Med. Vol. 30, pp 937-942 (1988).

7. Driscoll, K.E., Mauer, J.K., Crosby, L., and Windsor, D., "Relationship Between Alveolar Macrophage (AM) Activation, Lung Particle Clearance and Pulmonary Fibrosis," Toxicology Vol. 10, p 277 (1990).

8. Guide for the Care and Use of Laboratory Animals, NIH Publication 85-23, National Institute of Health, Washington, DC, 1985.

9. Deskin, R., Mosbergh, A., McNeill, K., Frazier, M., Pelroy, D., and Fischer, G., Tier I Generation and Characterization of Three Microcrystalline Waxes, Acrawax C, MP-12, Mekon White and Tier II Bacterial Mutagenicity and Mammalian Cell Cytoxicity Evaluation of the Collected and Particulate Samples, CRDC-CR-85037, U.S. Army Chemical and Research Development Center, Aberdeen Proving Ground, MD, July 1985, UNCLASSIFIED Report.

10. Thomson, S.A., Burnett, D.C., Crouse, C.L., Hilaski, R.J., and Muse, W.T., Acute Inhalation Toxicity of Pyrotechnically Disseminated Terephthalic Acid, CRDEC-TR-88134, U.S. Army Chemical Research, Development and Engineering Center, Aberdeen Proving Ground, MD, July 1988, UNCLASSIFIED Report.

11. Hanks, J.H., and Wallace, J.H., "Determination of Cell Viability," Proc. Soc. Exp. Biol. Med. Vol. 98, pp 188-192 (1985).

12. Gad, S.C., and Weil, C.S., "Statistics for Toxicologists," In Principles and Methods of Toxicology, pp 273-320, A.W. Hayes, Ed., Raven Press, New York, NY, 1984.

APPENDIX A

XM82 GRENADE COMPONENTS

1. Expulsion Charge (Propellant)

   - Class 6 Black Powder [Sulphur, Carbon (Charcoal), Potassium Nitrate]
   - MIL-P-223 (Black, Powder, Class 6)

2. Delay Detonator Assembly

   - Composition A-1A (Potassium Nitrate, Sodium Nitrate, Charcoal, Sulphur) MIL-P-22264 (Powder, Ignition, Gasless A-1A)
   - Lead Azide MIL-L-46225 (Lead Azide, RD-1333)
   - RDX (Cyclotrimethylenetrinitramine) MIL-R-398 (RDX, Type B)
   - Zirconium/Nickel Delay (Nickel, Zirconium, Barium Chromate, Potassium Perchlorate) MIL-C-13739 (Composition Delay Type III)

3. Transfer Lead

   - Plastic Bonded Explosive (PBX), Type I
   - HMX (Cyclotetramethylenetetranitramine), Copolymer of Vinylidene Fluoride and Hexafluoropropylene
   - MIL-E-81111 (Type I, Class 3)

4. Booster Lead

   - Composition CH-6 (RDX, Calcium Stearate, Graphite) MIL-C-21723

5. Burster Charge

6. Composition A-5 (RDX, Stearic Acid)

   - MIL-E-14970 (Explosive Composition A-5, Class 1 or 2)

7. Titanium Dioxide

Blank

APPENDIX B

ANIMAL HEALTH

DEPARTMENT OF THE ARMY
U.S. ARMY CHEMICAL RESEARCH, DEVELOPMENT AND ENGINEERING CENTER
ABERDEEN PROVING GROUND, MARYLAND 21010-5423

REPLY TO
ATTENTION OF

SMCCR-RST-V 14 December 1990

MEMORANDUM FOR Dr. Thomson

SUBJECT: Inspection of Animal Shipment

Dr. Thomson:

On 6 December 1990 I examined the shipment of rats from Charles River, Order # 1421393 that arrived on 5 December 1990. The approximate date of birth of these animals is 5 October 1990. All animals appeared normal. Cultures of tracheal washes and gastrointestinal tract were performed by USAMRICD. No growth was found on tracheal wash cultures and no enteric pathogens were isolated from the gastrointestinal tracts.

These animals meet the criteria for utilization on your protocol.

Billy W Howard

BILLY W. HOWARD
MAJ, VC
Chief, Veterinary
Services Branch

DEPARTMENT OF THE ARMY
U.S. ARMY CHEMICAL RESEARCH, DEVELOPMENT AND ENGINEERING CENTER
ABERDEEN PROVING GROUND, MARYLAND 21010-5423

REPLY TO
ATTENTION OF

SMCCR-RST-V

18 January 1991

MEMORANDUM FOR Dr. Thomson

SUBJECT: Inspection of Animal Shipment

Dr. Thomson:

We have received results from the cultures of the two week post-receipt of the shipment of rats from Charles River, Order # 1421393 that arrived on 5 December 1990. The approximate date of birth of these animals is 5 October 1990. Cultures of tracheal washes and gastrointestinal tract were performed by USAMRICD. No respiratory pathogens were found on tracheal wash cultures and no enteric pathogens were isolated from the gastrointestinal tracts.

These animals meet the criteria for utilization on your protocol.

Billy W. Howard
BILLY W. HOWARD
MAJ, VC
Chief, Veterinary
Services Branch

Blank

APPENDIX C

HISTOPATHOLOGY SUMMARY

This report has been renumbered to coincide with this report.

PAI Pathology Associates, Inc.

Suite I
15 Worman's Mill Court
Frederick, Maryland 21701
Phone: (301) 663-1644
FAX: (301) 663-8994

PATHOLOGY REPORT
FOR

ACUTE INHALATION TOXICITY EFFECTS OF EXPLOSIVELY DISSEMINATED TITANIUM DIOXIDE (XM82 GRENADE) 24-HOUR AND 14-DAY SACRIFICES

PROTOCOL NUMBER 22091000A265

TABLE OF CONTENTS

PATHOLOGY REPORT

Acute Inhalation Toxicity Effects of Explosively Disseminated Titanium Dioxide (XM82 Grenade) 24-Hour and 14-Day Sacrifices

Protocol No. 22091000A265

INTRODUCTION

This report by Pathology Associates, Inc. (PAI) for Toxicology Division, Research Directorate, Chemical Research Development and Engineering Center (CRDEC), Aberdeen Proving Ground, MD 21010-5423, presents the results of gross and microscopic examination of tissues from rats exposed via inhalation to various concentrations of explosively disseminated titanium dioxide (TiO_2) dust and sacrificed 24 hours or 14 days post-exposure (PE) in accordance with Protocol No. 22091000A265. The pathology evaluation was conducted under the provisions of Contract No. DAAA15-88-D-0012.

EXPERIMENTAL DESIGN AND METHODS

Male Fischer 344 rats were exposed by nose-only inhalation to one of three concentrations of TiO_2 dust for 30 minutes. Air-exposed and fuse/fuel-exposed rats served as controls. Twenty-four hours or fourteen days PE the rats were euthanized via asphyxiation with carbon dioxide and necropsied in accordance with PAI SOPs and contract requirements. The experimental design for animals designated for pathologic evaluation is as follows:

EXPERIMENTAL DESIGN

DOSE GROUP	NUMBER OF ANIMALS	
	24 Hr. (PE) Sacrifice	14 Day (PE) Sacrifice
Air-exposed Control	6	6
Fuse/Fuel-exposed Control	6	6
TiO_2 (Low Concen.)	6	6
TiO_2 (Medium Concen.)	6	6
TiO_2 (High Concen.)	6	6

Tissues required by contract were fixed in 10% neutral buffered formalin for at least 48 hours. For the Air-exposed Control and High dose groups, all tissues required by contract (full histopathology) were processed through paraffin, sectioned, at approx. 6 µm, stained witn hematoxylin and eosin, and examined microscopically. For the remainder of the groups, as required by contract, limited tissues consisting of lung, larynx, trachea, tracheobronchial lymph node(s) (TBLN), liver, kidneys, and nose (4X) were prepared and evaluated.

RESULTS AND DISCUSSION

Gross Necropsy Findings

Discoloration of the lungs (dark) was noted in at least one rat from each of the groups except the fuse/fuel-exposed group necropsied at 24 hours PE. Many of these lesions were due to congestion caused by carbon dioxide asphyxiation. No treatment-related gross lesions were noted in the rats necropsied at 14 days PE.

Histopathology

Microscopic findings are presented in tabular format in Sections II and III. Summary data are presented by dose group in the Project Summary Tables, while individual diagnoses are presented in the Tabulated Animal Data Tables. The Reports Code Table (Appendix 1) defines the symbols for distribution and severity used in the tables. Abbreviations used in any of the tables are explained in Appendix 2.

The administration of explosively disseminated TiO_2 dust resulted in the deposition of brown granular pigment in the lungs of all exposed rats. The pigment was contained within macrophages. No toxic or inflammatory reactions other than phagocytosis of the dust particles in the lung were present. Pigment-laden macrophages were also present in the lungs of all rats in the fuse/fuel-exposed control group. A very small number of pigmented macrophages were present in the TBLN of 3/6 high dose rats at 14 days PE. There were no other significant gross or histopathologic differences between the animals in the 24 hour PE and 14 day PE groups.

24-Hour Sacrifice

In all of the TiO_2-exposed rats sacrificed at 24 hours PE, finely-granular brown pigment was present throughout the lung within small airways (terminal and respiratory bronchioles) and alveoli. Occasionally, pigment-laden macrophages were present in the larger bronchioles or bronchi. All of the pigment was intracellular, having been phagocytized by alveolar macrophages. The pigment elicited no inflammatory reaction other than the presence of the pigment-laden alveolar macrophages. While the number of pigmented macrophages increased from the low to high dose group, the severity of the histiocytic response in all animals was minimal. A very small number of pigmented macrophages were also present in the lungs of rats in the fuse/fuel-exposed control group. The amount of granular brown pigment in these macrophages was extremely small. No pigment or pigmented macrophages were present in the TBLN.

14-Day Sacrifice

Like those animals sacrificed at 24 hours PE, the lungs of all rats exposed to TiO_2 dust contained finely-granular brown pigment within alveolar macrophages. The severity and distribution of the histiocytic response was indistinguishable from the corresponding groups sacrificed 24 hour PE. No inflammatory reaction other than the response of the pigment-laden macrophages was present in the lungs. Fuse/fuel-exposed rats also had changes identical to those in the fuse/fuel-exposed rats sacrificed at 24 hours PE.

TBLN from three of the high dose rats contained very small numbers of pigmented macrophages. This change was not present in any other dose groups.

Miscellaneous

Several other incidental lesions were present, only two of which warrant further comment. Multifocal, centrilobular, hepatocellular cytoplasmic vacuolization was noted in many of the rats. No relationship to treatment was apparent. This change probably reflected the presence of glycogen and was not considered a significant pathologic change.

Respiratory epithelial hyperplasia was present in at least one rat from each dose group except the low dose group sacrificed at 24 hours PE and in at least two rats from each dose group sacrificed at 14 days PE. No relationship to treatment was apparent. The change was minimal in most affected rats and occurred along the dorsolateral portion of the meatus (dorsal to the root of the incisor) in the most rostral nasal section. This change was most likely due to exposure to an unknown toxic or infectious agent. Similar lesions have been noted in rats from numerous test facilities, and no relationship to infectious or toxic agents have been proven (personal observation).

SUMMARY AND CONCLUSIONS

Male Fischer 344 rats were exposed by inhalation to one of three concentrations of explosively disseminated TiO_2 dust for 30 minutes. Air-exposed and fuse/fuel-exposed rats served as controls. Twenty-four hours or fourteen days PE the rats were euthanized via asphyxiation with carbon dioxide and necropsied.

Microscopically, the only significant treatment-related finding was the presence of pigment (primarily within macrophages) in the alveoli and small airways of rats exposed to both TiO_2 and fuse/fuel necropsied at 24 hours PE and 14 days PE and in the tracheobronchial lymph nodes of 3/6 high dose rats necropsied at 14 days PE. No other significant morphologic changes were present.

Lucas H. Brennecke, D.V.M.
Diplomate, ACVP
February 20, 1990

QUALITY ASSURANCE

This study was examined for compliance with Good Laboratory Practices as published by the U. S. Environmental Protection Agency in 40 CFR Part 792 (effective 17 Aug 1989). The dates of all inspections and the dates the results of those inspections were reported to the Study Director and management were as follows:

Phase inspected	Date	Date reported
Dosing via inhalation	18 Dec 1990	20 Dec 1990
Lavage, chemistry, necropsy	3 Jan 1991	4 Jan 1991
Pathology Report	25 Mar 1991	25 Mar 1991
Data & Final Report	10 July 1991	11 July 1991

To the best of my knowledge, the methods described were the methods followed during the study. The report was determined to be an accurate reflection of the raw data obtained.

Dennis W Johnson

DENNIS W. JOHNSON
Quality Assurance Coordinator, Rsch 17 July 91